K-ras Mutation in Focal Proliferative Lesions of Human Pancreas

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Abstract

The K-ras gene is mutated in 75% of human pancreatic adenocarcinomas and in a number of hyperplastic ductal lesions from noncarcinoma patients. In this study, the incidence of K-ras mutation was determined in a spectrum of focal proliferative pancreatic lesions to evaluate their preneoplastic significance. PCR-based mutation-enriched RFLP analysis was used to identify mutations in codon 12. Immunostaining for Ki67 and p53 was also performed. Forty-seven percent of intraductal nonpapillary hyperplasias (8 of 17) contained codon 12 mutations, as did 55% of adenomatoid hyperplasias (6 of 11). This rate increased to 61% in papillary hyperplasias (27 of 44) and to 78% when there was severe dysplasia (7 of 9). The fraction of cells staining for the Ki67 proliferation marker showed a general correlation with the rate of K-ras mutation. Nuclear staining for p53 protein was seen only in two ductal lesions with severe dysplasia. No mutations were found in normal acinar tissue (n = 38), squamous metaplasia (n = 13), ductal complexes (n = 8), or focal acinar cell dysplasia (n = 5). There seemed to be a general correlation of proliferative potential with the presence of K-ras mutation in ductal lesions. However, because of the high prevalence of lesions with K-ras mutations, we conclude that this mutation alone cannot be taken as proof of significant risk for progression to carcinoma. Efforts to use the presence of K-ras mutations in DNA harvested from pancreatic juice or duodenal aspirates as an approach for diagnosis of occult pancreatic carcinoma seem vulnerable to a high false-positive rate.

Introduction

Carcinoma of the pancreas remains a disease that is infrequently diagnosed early enough to allow successful treatment (1). It ranks just below carcinomas of the lung, colon, breast, and prostate as a cause of death from cancer in the United States (2). This highlights the need for improved approaches to prevention and early diagnosis. The former requires understanding of etiology, and the latter requires knowledge of early steps in tumor development.

It is striking that activation of the K-ras oncogene is present in 75-90% of pancreatic carcinomas, when composite series are considered (3-6). Activation is nearly always due to a mutation at position 1 or 2 of codon 12. Because approximately 81% of carcinomas of the pancreas are of a ductal phenotype (7), it seems reasonable to assume that these data are derived primarily from ductal carcinomas. Our finding that six of eight small ductal adenocarcinomas with maximum diameters in the range of 1-3 cm contained mutated K-ras (8) suggests that small and large carcinomas have a similar mutation rate. This supports the idea that the K-ras mutation is an early rather than a late change in most carcinomas.

In hamsters, mutations in K-ras codon 12 are present in the majority of N-nitrosobis(2-oxopropyl)amine-induced ductal adenocarcinomas (9-11). Some of the putative preneoplastic intraductal proliferative lesions in hamsters, including 26% of nonpapillary hyperplasias, 46% of papillary hyperplasias, and 76% of carcinomas in situ also contain codon 12 mutations in K-ras (11). Similar data are reported for hamsters treated with N-nitroso(2-hydroxypropyl,2-oxopropyl)amine (12), with mutations found in 50, 90, and 100% of nonpapillary, papillary, and atypical hyperplasia/carcinoma in situ lesions, respectively.

These observations and the high rate of K-ras mutation in human pancreatic carcinomas suggest that the presence of such mutations could be used as a partial basis for assessing the neoplastic potential of focal proliferative lesions in the human pancreas. This reasoning implies that preneoplastic significance should be assigned whenever the mutation is found in a lesion of the pancreatic ductal epithelium. The classification (nomenclature) and criteria for focal proliferative and metaplastic lesions in human pancreas have been described (13-16). The lesions that are relevant to this study are listed in Table 1. There are now several reports of K-ras mutation in ductal hyperplasia, although the rates reported have varied from 0 to 66% in various studies (see “Discussion”).

In this study, we have isolated DNA from a group of focal proliferative and metaplastic pancreatic lesions in FFPE tissue from autopsies or surgically resected pancreateces. Most lesions were harvested from pancreases without carcinoma or pancreatitis. This DNA was used for mutational analysis of codon 12 of the K-ras gene. Immunostaining for Ki67 and p53 was also performed on sections from these specimens. We have included adenomatoid ductal hyperplasia, squamous metaplasia, ductal complexes, and acinar cell foci, categories for which data have not been reported previously. The results we report are relevant to the etiology of carcinoma of the pancreas and bear directly on approaches that have been suggested for the early diagnosis of pancreatic cancers.

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Materials and Methods

Tissue. Twenty-four of the pancreases were from routine autopsies of patients without pancreatic carcinoma (Table 2), including tissues stored from a previous project, in which 12 blocks were taken from each pancreas (17). Twenty-one of the pancreases were surgically resected for pancreatic disease (suspected pancreatic carcinoma or chronic pancreatitis; see Table 2), and of these, only 3 harbored carcinomas with \( K-ras \) mutation. Only two of the eight ductal hyperplastic lesions harvested from these three pancreases contained the same mutation as the carcinoma, so we assumed that intraductal extension of carcinomas had little impact on our data.

Lesions were classified as shown in Table 1. Here, we use “focal proliferative lesion” in a generic sense to indicate all seven categories listed in Table 1.

To evaluate the multiplicity of ductal hyperplastic lesions, foci were counted on slides from 10 pancreaces, for which 6–12 blocks were available for each. Close-lying foci that appeared likely to represent a single lesion within a branching duct were counted as one. However, it is possible that some foci in adjacent blocks represent the extension of a single lesion through the duct system. Thus, the counts are regarded as approximations.

Immunohistochemistry. Sections from 10 of the pancreases were stained, using the avidin-biotin complex immunoperoxidase method, with the MIB-1 antibody (Immunotech, Westbrook, ME) for the evaluation of the Ki67 cell proliferation marker and with DO7 (BioGenex Laboratories, San Ramon, CA) for the evaluation of p53 protein. An additional 11 pancreaces were stained using only the MIB-1 antibody. The DO7 antibody was chosen because of its superiority in detection of p53 in FFPE tissues (18). MIB-1 antibody was used as a 1:50 dilution of the monoclonal antibody. DO7 antibody was used as a 1:40 dilution of the primary mouse monoclonal antibody. Sections stained without the primary antibody were used as a negative control. Slides containing sections of a breast carcinoma served as the positive control for p53 and lymph node was used for Ki67. Antigen retrieval was used to maximize the effectiveness of immunohistochemistry in FFPE tissues. Slides were placed in a Heat-induced Epitope Retrieval solvent (Ventana Medical, Tucson, AZ) using a capillary gap system and exposed to the solvent in a preheated steam chamber for 20 min and then cooled before staining. Nuclear staining that was clearly above background was required to mark a cell as positive for Ki67, and nuclear staining in ≥10% of a cell population was required to establish positive staining for p53. Cytoplasmic staining was ignored.

DNA Isolation from Paraffin Blocks. Following comparison to corresponding H&E-stained sections, lesion-containing regions of unstained sections were collected, and DNA was isolated using a method based on a previously described method (19). A 20-μm-thick paraffin-embedded section was melted onto a slide (not deparaffinized), and the area of interest was identified using a dissecting microscope by comparison to the adjacent H&E-stained 5-μm section. The area of interest (size range = 0.5–2 mm in diameter) was microdissected using a clean, autoclaved needlepoint. The loosened fragments were collected on a 3% agarose cube and transferred to a microfuge tube for digestion with protease K [at 55°C for 16 h in 50 μM Tris-HCl (pH 8), 1 mM EDTA, 0.5% Triton X-100, and 200 μg/ml protease K] followed by heat inactivation of the pro-
tenase (at 99°C for 10 min). Precautions against cross-contamination of samples were observed.

**meRFLP Analysis.** The first amplification consisted of a 5–10-min denaturation at 94°C, followed by 15 cycles of 48 s at 94°C, 48 s at 52°C, and 1 min at 72°C, followed by a final extension of 10 min at 72°C. Reaction conditions were as follows: 2 units of AmpliTaq or AmpliTaq Gold and its corresponding buffer (Perkin-Elmer Corp., Foster City, CA), 40 pmol of each primer (BstNI-R and BstNI-5'), 20 μM each deoxynucleotide, and 1 mM MgCl₂ in a total of 40 μL. This amplification involved a mismatched primer that generates a BstNI recognition sequence in those products with wild-type codon 12 (19–21). This amplification was followed by heat denaturation of the polymerase (at 99°C for 5 min) and elimination of the majority of wild-type sequences by BstNI digestion according to the manufacturer’s recommendations (20 μL of PCR product in a 40-μL reaction at 60°C for 2 h; New England Biolabs, Beverly, MA). The second amplification of predominantly mutated products (1 μL of the digest) was similar to the first amplification, with the following exceptions: ULTma polymerase and its buffer (Perkin-Elmer Corp.) were used, primers were BstNI-1' and BstNI-3', 30 cycles were performed, and the polymerase denaturation was for 20 min at 99°C. This amplification was followed by a second BstNI digestion (20 μL of PCR product in a 40-μL reaction at 60°C for 16 h) to distinguish mutated products from carry-over wild-type products. Digested products were resolved on a 1% agarose gel.

**DNA Sequence Analysis.** The modified dideoxy chain termination method of Sanger et al. (22) was used for DNA sequence analysis according to the AmpliCycle protocol or the ABI PRISM dye terminator cycle sequencing kit (Perkin-Elmer Corp.).

**Statistical Analysis.** Rates of K-ras mutation were calculated for each type of lesion. Because of the small numbers of lesions and several lesion types with no observed mutations, “exact” statistical methods, appropriate for these conditions, were used to calculate 95% confidence intervals. A global comparison among the proliferation rates in different lesion groups and the comparison of the rates of specific mutations were made using exact methods (Fisher’s exact test). Mutation rates in various groups were compared using the χ² test. These methods were implemented using the statistical package StatXact (Cytel Software, Cambridge, MA).

**Results**

**Histological Evaluation.** In selecting lesions for study, it was not always possible to select “classic” lesions with the characteristics of only one of the established categories of ductal hyperplasia. Several ductal lesions included distinct areas of both nonpapillary and papillary hyperplasia that were so intimately mixed that they were not separable by microdissection. These four lesions are included with papillary hyperplasia in Table 1. Similarly, 9 of 11 examples of adenomatoid ductal hyperplasia were intimately associated with either nonpapillary or papillary ductal hyperplasia (Fig. 1). The microdissected samples from these lesions included hyperplastic epithelium from both adenomatoid ductules and adjacent ducts.

There were 91 foci of ductal hyperplasia in 107 slides from the 10 pancreases that were counted to evaluate multiplicity (range = 1–17 foci per pancreas).

**K-ras Evaluation.** Fig. 2 is representative of typical meRFLP analysis of K-ras codon 12. The complete results are summarized in Table 1. No mutations were found in 13 squamous metaplasias, 8 ductal complexes, 5 focal acinar cell dysplasias, or any of the samples of acinar tissue or other normal tissues. The rate of mutation was higher in lesions with papillary ductal hyperplasia (61%) than in those with nonpapillary ductal hyperplasia (47%), although the differences were not significant. The mutation rate for adenomatoid ductal hyperplasia (55%) was between the rates for papillary and nonpapillary ductal hyperplasia. This was expected because the majority of these lesions were mixed with either papillary or nonpapillary ductal hyperplasia. The highest rate of mutation (78%) was found in lesions that showed dysplasia (atypical hyperplasia). The rate of mutation in lesions with papillary hyperplasia and atypical hyperplasia was significantly higher than that in normal tissues, squamous metaplasia, or ductal complexes. A global comparison of rates in lesions and normal tissues was statistically significant (P = 0.0001; Fisher’s exact test). The ratios of specific mutations in the ductal hyperplasias were (wild type = GGT): GTT, 53%; GAT, 29%; CGT, 14%; and TGT, 4% (n = 49; one of the 48 mutated lesions contained two different mutations).

The majority of lesions with atypical hyperplasia were from cases that harbored carcinomas, so we cannot rule out the possibility that some of the lesions were intraductal extensions of a ductal carcinoma. However, of the seven atypical hyperplasias with mutations, only two contained the same mutation as the corresponding carcinoma.

A higher mutation rate was observed in cases with pancreatic carcinoma versus cases without pancreatic disease (P = 0.002, Fisher’s exact test; Table 2). This is reflected in the higher (not significant) percentage of mutated lesions in surgical versus autopsy cases. The mutation rate in cases with chronic pancreatitis or with biliary or ampullary carcinomas was similar to that in cases without pancreatic disease.

We found that recently harvested pancreases in which we carefully limited the period of fixation in formalin to 4–6 h provide a more reliable source of DNA for amplification than the majority of pancreases from an earlier autopsy series (data not shown). Surgical pathology specimens are less likely to have been fixed for long periods and have provided a better
source of DNA than have pancreases from many of the older autopsies.

**Immunostaining.** Two autopsy pancreas sections showed no positive staining in any cell type with the MIB-1 antibody, and nine others showed only faint staining. These were dropped from further evaluation because of the probability that the lack of staining reflected loss of reactive epitope due to fixation. A few cells were labeled by the Ki67 antibody in 5 of 17 cross-sections of normal pancreatic ducts. The ducts were scored as positive when 1–5% of the cells showed nuclear staining.

Nine of 12 ducts with nonpapillary hyperplasia showed labeling. Labeling was present in 17 of 18 ducts with papillary hyperplasia. In ducts with papillary hyperplasia, the positive nuclei were frequently located in the basal portion of papillary projections (Fig. 3). Rare mitotic figures were encountered in papillary hyperplasia but not in nonpapillary areas or adenomatoid areas. Labeling was noted in four of six foci of adenomatoid ductal hyperplasia and was more commonly noted in glands lined by polygonal cells with vesicular nuclei than it was in glands that showed pyloric gland metaplasia. Ductal complexes showed scattered Ki67-positive epithelial cells. Only one of three foci of squamous metaplasia was labeled. All 11 transections of ducts with atypical hyperplasia showed labeling that was present in a higher fraction of cells than that noted in hyperplasias, and labeling was present in 25–50% of cells in one specimen. Thus, the fraction of positive foci rose progressively in normal ducts, adenomatoid hyperplasia, nonpapillary hyperplasia, papillary hyperplasia, and atypical hyperplasia. The labeling index was highest in atypical hyperplasia.

Not all lesions present in these sections had been micro-dissected for evaluation of the status of c-K-ras, but correlation of Ki67 staining and c-K-ras mutation was possible in 18 lesions. Ten of 11 lesions with mutation were positive for the proliferation marker, whereas four of seven lesions with wild-type c-K-ras showed the marker. Although this difference is not significant (Fisher’s exact test), it appears that lesions with mutant c-K-ras have a higher rate of proliferation.

Nuclear staining for p53 protein was noted in foci of atypical hyperplasia of the ductal epithelium in two pancreases with this lesion but not in ducts with nonpapillary (n = 11), papillary (n = 7), or adenomatoid (n = 4) hyperplasia or in 11 transections of normal duct. Ductal adenocarcinomas were present in both of the pancreases with p53 staining in ducts with atypical hyperplasia. One transection of a normal main pancreatic duct showed nuclear staining in 25–50% of the epithelial cells. We have previously reported occasional examples of positive p53 nuclear staining in apparently normal parenchymal cells (23).

**Discussion**

The data reported above document a significant rate of K-ras mutation in nondysplastic hyperplastic lesions of pancreatic ductal epithelium. We are not aware of prior evaluations of lesions in the categories of focal acinar cell dysplasia, ductal squamous metaplasia, adenomatoid ductal hyperplasia, and ductal complex. There are multiple independent reports of K-ras mutation in nonpapillary and papillary hyperplasia and atypical ductal hyperplasia (Table 3; Refs. 24–32). The rate of mutation within these categories of lesions varies widely from study to study, but the number of reports provides strong support for the occurrence of such mutations. The rate of mutation is clearly highest in atypical hyperplastic lesions from patients that have carcinoma of the pancreas. This may partially reflect intraductal invasion by carcinomas but is equally consistent with a precursor role for the lesions.

Although we found an unusually high fraction of lesions with GTT mutations, the ratio of specific lesions in our series is not significantly different from a composite series of mutations assembled from the reports cited in Table 3 (P = 0.06, Fisher’s exact test). However, we found significantly more GTT and fewer GAT mutations than are reported in a combined series of mutations in carcinomas [GTG, 30%; GAT, 47%; CGT, 16%; TGT, 5%; GCT, 1%; AGT, 1% (n = 469); Refs. 5, 29, 31, 33–37].
Nonpapillary and papillary ductal hyperplasia often occur in the same pancreas, and sometimes, they occur in continuity. Intuitive speculation regarding the consequences of proliferation of a lining epithelium in a confined space suggests that flat hyperplasia would precede papillary hyperplasia. According to the data shown in Tables 1 and 3, lesions of both patterns may contain K-ras mutations. We conclude that these are closely related and sequential lesions. Our data suggest that the rate of K-ras mutation is higher in papillary than in nonpapillary lesions. These observations are consistent with an association of increased proliferative potential with mutation of K-ras. The data also indicate that ductal hyperplasia can occur independently of K-ras mutation.

In our study, adenomatoid ductal hyperplasia was usually closely associated with ductal hyperplasia and had a K-ras mutation rate that was intermediate between those of nonpapillary and papillary hyperplasia. We conclude that adenomatous hyperplasia represents extension of ductal hyperplasia into small ductules and lobules.

Ductal complexes were not closely associated with ductal hyperplasia. We regard ductal complexes as likely to arise as a result of either metaplasia of acinar cells or proliferation of centroacinar cells, to replace acinar cells within acinar lobules. The fact that no ductal complex contained K-ras mutation is consistent with a different origin than that for ductal hyperplasia and adenomatoid lesions.

In the colon, the pathway for progression of lesions to carcinoma is well defined (38). In this model, a lesion acquires a new mutation and then progresses to the next stage. Jen et al. (39) have shown that the order in which these mutations occur can have an impact on both the morphology of the lesion and its malignant potential. They found that mutation of K-ras was found in 19 of 19 nondysplastic ACFs but not in the 1 dysplastic ACF. Mutation of the APC gene was found only in the dysplastic ACF not in the 19 nondysplastic ACFs. They present a model suggesting that, if the initiating event of an ACF is mutation of K-ras, it is likely to remain nondysplastic and should not progress further. However, if the initiating event is mutation of the APC gene, it is likely to develop into a dysplastic ACF and progress to adenoma or carcinoma, if it acquires mutations in other genes, including K-ras.

Spruck et al. (40) have described a situation in transitional cell carcinomas of the bladder in which the order of mutations defines the type of lesion, although both may progress to an invasive carcinoma. In this case, early deletions of chromosome 9 (including the p16 gene) lead to noninvasive papillary tumors. Subsequent mutation of p53 can result in invasiveness. However, dysplastic/carcinoma in situ lesions often already contain mutation of p53 and result in nonpapillary invasive carcinomas. In this case, deletions of chromosome 9 may occur subsequent to p53 mutation (40, 41).

These studies show that the sequence of mutation acquisition and not just the accumulation of mutations can be very important in determining the potential for progression of preneoplastic lesions. Furthermore, the fact that perhaps 10–25% of pancreatic carcinomas have wild-type K-ras reinforces the view that molecular changes other than K-ras mutation can be anticipated in some preneoplastic lesions. Positive immunostaining for p53 protein has been reported previously in ductal hyperplasia (31) and carcinoma in situ. The fact that Boscman et al. (42) found strongly positive staining in 3 of 12 examples of ductal hyperplasia (without atypia) may reflect the fact that all their material was from patients with carcinomas, whereas our study material was not, or it may, perhaps, reflect a difference in criteria for classification of ductal lesions. We regard their “without atypia” figure (Fig. 3 in Ref. 42) to show significant dysplasia. Our data are consistent with the view that K-ras mutation can be an early event during carcinogenesis in the pancreas, whereas p53 mutation appears to occur at a later stage.

Tada et al. (29) and Moskaluk et al. (31) concluded that K-ras mutations in pancreatic ductal hyperplasia cannot be taken, in isolation, as proof of a significant increased risk for progression to carcinoma. Our data support this conclusion. Moskaluk et al. (31) suggest that the presence of a mutation in the p16 gene is a better indicator of risk of progression to cancer in preneoplastic lesions.

It has also been suggested that evidence of K-ras mutation would support a diagnosis of carcinoma for biopsy or cytological specimens that have been classified as suspicious but are inadequate for a definitive diagnosis (43, 44). However, in view of the frequency of K-ras mutation in ductal hyperplasias, in apparently benign tumors, and in cases of chronic pancreatitis, the utility of K-ras mutation in the diagnosis of malignancy, based on DNA harvested from pancreatic juice (45), duodenal aspirates (46, 47), or feces (28), must be questioned. After finding K-ras mutation but no evidence of cancer for 78 months of follow-up in 20 cases of chronic pancreatitis, Furuya et al. (48) concluded that the presence of K-ras mutation in the duodenal juice of patients with chronic pancreatitis has little

Table 3 Summary of reported K-ras mutations in ductal hyperplasias a

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<th>Study</th>
<th>Method</th>
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<th>Mixed</th>
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a ASO, allele-specific oligonucleotide hybridization; AS ligation, allele-specific ligation analysis; sequencing, DNA sequence analysis of PCR products; FH, flat (non-papillary) ductal hyperplasia; PH, papillary ductal hyperplasia; AH, atypical ductal hyperplasia in pancreas with no evidence of carcinoma; AH/CA, atypical ductal hyperplasia in pancreas with carcinoma; mixed, unspecified or combinations of nonpapillary and papillary hyperplasias.
predictive value for cancer risk. This conclusion is supported by the findings of Uehara et al. (47) of K-ras mutation in duodenal juice aspirates from 4 of 13 patients with chronic pancreatitis and 2 of 10 patients without clinical evidence of a pancreatic disorder.

Although intraductal papillary-mucinous adenomas and borderline tumors are regarded as a separate group of neoplasms of low or uncertain malignant potential, some have been reported to progress to invasive ductal or noncystic mucinous carcinomas (36, 49). About half of the intraductal tumors have been reported to harbor K-ras mutation (24, 36, 50–52). It follows that about half of these would be missed by screening based on detection of K-ras mutation in pancreatic secretions. It should be noted that we have encountered some problems using the meRFLP technique on very small samples. During the early phase of establishing the methods used in this study, a number of false-positive results were generated due to misincorporation errors of Taq polymerase. Because of the small amount of quality starting material, a large number of amplification cycles was needed to visualize the products on agarose gels. This alone can lead to base misincorporation by Taq polymerase (error rate = 1 × 10⁻⁵, manufacturer’s data; Perkin-Elmer Corp., Foster City, CA), but the problem was exacerbated by a number of conditions. Because one of the primers has a mismatch in it, a polymerase with a proofreading function could not be used in the first amplification step. Also, the two-step protocol includes a very strong selection for mutant DNAs, increasing the chances of detecting an incorporation error. Finally, because of the small amount of starting material, an early error would represent a large proportion of the sample. To address these issues, we have made a number of modifications to our protocols. First, we are using more starting material in both amplification steps. Also, to minimize the chances of a Taq error, we have reduced the number of amplification cycles in the first step of the procedure and have reduced the concentration of nucleotides in our amplification buffer. To enhance digestion, we now heat-denature the Taq protein before digestion to eliminate any steric hindrance it may create by remaining bound to the DNA. We also use a polymerase with a proofreading function in the second amplification step.

Several possible pathways have been proposed for the development of pancreatic ductal carcinoma. The traditional view has been that ductal adenocarcinomas arise via a dysplasia-carcinoma sequence that occurs in main or branch ducts (13, 53). An alternate view, based on study of the hamster model, suggests that small intrabiliary ductules are involved (54) and that these ductules sometimes develop within islets (55). More recently, an adenoma-carcinoma sequence has been proposed as the origin of some ductal and noncystic mucinous adenocarcinomas. The adenoma stage is termed intraductal papillary-mucinous adenoma in the revised WHO classification of pancreatic carcinomas (56). Squamous metaplasia does not play a role in any of these pathways, and it has not been regarded as a preneoplastic lesion in evaluations based on morphology (13). Similarly, ductal complexes are not part of either pathway. The failure to detect K-ras mutation in these two lesions is supportive of this assessment.

Consideration of the prevalence of the lesions and the incidence of K-ras mutations suggests that, at most, a small fraction of the ductal hyperplastic foci progress to carcinoma, even when K-ras mutation is present. On the basis of several conservative assumptions, it can be calculated that the fraction of foci of ductal hyperplasia with K-ras mutation that progress to carcinoma is <1% (see “Appendix”).

In conclusion, we agree with Tada et al. (29) and Moskaluk et al. (31) that the finding of a codon 12 K-ras mutation in a focal proliferative lesion of the human pancreas is not a sufficient basis for assigning premalignant status to the lesion. Although K-ras mutation is certainly involved in the development of the majority of pancreatic cancers, mutation of other genes is also vital to their development. Nonetheless, if we assume that papillary ductal hyperplasia reflects a higher proliferation potential than the nonpapillary hyperplasias (flat ductal hyperplasia and adenomatoid ductal hyperplasia), then we see a general correlation of proliferative potential with the rate of K-ras mutation. The highest rate of mutation was noted in lesions with significant dysplasia (atypical hyperplasia).

References


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